THE SEXUAL FORMS OF THE MALARIAL PLASMODIA OCCURRING IN THE BLOOD OF MAN *

CHARLES F. CRAIG, M.D.
WASHINGTON, D. C.

The forms of the malarial plasmodia concerned in sporogony which can be differentiated in the blood of the human host are of great interest from both a practical and theoretical standpoint. The presence of these sexual forms, which are intended to complete their development within the mosquito, proves beyond question that the patients in whom they occur are infective to these insects, and that they are thus true "carriers" of malarial disease. The demonstration of these forms is, therefore, of importance in malarial epidemiology and prophylaxis, and as but very brief descriptions of the sexual forms are given in most English and American works dealing with the malarial fevers or the protozoa, it has appeared to me that a detailed account of their morphology, together with a discussion of certain points concerning their rôle in epidemiology, might prove of interest and value to those interested in the diagnosis and prophylaxis of the malarial fevers.

As is well known, there occur in the blood of man, besides the forms of malarial plasmodia concerned in the human cycle of development, or schizogony, certain other forms which are intended to develop only within the mosquito. The observations of Schaudinn¹ regarding parthenogenesis of the macrogamete within man have not been confirmed by many observers, and I, although I have most carefully examined many cases, have not been able to demonstrate the forms described by Schaudinn as parthenogenetic forms. Therefore, in the absence of more definite proof than we yet possess, I believe that it is the part of wisdom to consider the question of parthenogenesis as an open one, and that the sexual forms of the malarial plasmodia occurring in the blood of man are capable of development only within the mosquito.

Of the sexual forms, the male are known as microgametocytes and the female as macrogametes; while both forms are generally referred to as gametes or gametocytes, when spoken of collectively. While no true devel-

---

* This report by Dr. Craig, Captain, Medical Corps, U. S. Army, is of work done in the Laboratory of the Surgeon-General's office and is published with permission of the Surgeon-General of the Army.

1. Schaudinn, F.: Studien über krankheitserregende Protozoen, Arb. a. d. k. Gsndhsamte, 1902, xix, 169.

opment of these bodies occurs within man, if the blood containing them be removed from the body, the microgametocytes frequently undergo flagellation, and produce free, motile, filamentous forms, known as microgametes. In very rare instances individual microgametes may be observed to penetrate a macrogamete, representing a process of fertilization normally occurring within the middle intestine of the mosquito.

The differentiation of the sexual forms or gametes is not a very difficult matter, although the opposite opinion appears to be generally prevalent. The estivo-autumnal gametes, because of their crescentic shape when fully developed, are easily recognized, even by a novice in malarial parasitology, but the intracorpuseular stages of the development of the gametes of all of the species of plasmodia are more difficult of recognition, although with a clear knowledge of their morphology and a little practice, all of their stages of development may be differentiated in stained specimens of blood with the exception of the very earliest intracorpuseular stage, in which the morphology is still a matter of dispute. The fully developed gametes of *Plasmodium vivax* (the tertian plasmodium) and *Plasmodium malariae* (the quartan plasmodium) are easily differentiated when one is thoroughly acquainted with their morphology.

In discussing the sexual forms of the malarial plasmodia found in man, it is necessary to consider the gametes in general, and the macrogametes, microgametocytes, and microgametes, of each species, in particular.

THE RELATIVE PROPORTION OF MALE AND FEMALE GAMETES

But few observations have been made on the relative proportion of microgametocytes and macrogametes which occur in the peripheral blood of man. As these forms may be readily differentiated, it is rather surprising that the observations upon this phase of our subject are so limited, the best being those of Stephens and Christophers² and Ruge.³ Stephens and Christophers studied the subject in estivo-autumnal infections and found the proportion to be 53 microgametocytes (male) to 33 macrogametes (female); while Ruge, in a large number of benign tertian infections found that the relative proportion varied greatly in different individuals, in some there being practically equal numbers of male and female gametes, while in others only one microgametocyte was found to 50 macrogametes.

2. Stephens, J. W. W., and Christophers, S. R.: The Agglutination of Sporozoites, Rep. Malarial Com. Roy. Soc. Lond., 1889-1900, Series 3, p. 1.

3. Ruge, R.: Fragen und Probleme der modernen Malariaforschung, Centralbl. f. Bakteriöl. u. Parasitenk., 1902, xxxii, 776.

I have frequently observed instances of estivo-autumnal infection in which the proportion of microgametocytes to macrogametes was as 50 to 5; and I believe that in this type of infection the male form always outnumbers the female, and that the same is probably true in tertian and quartan infections.

Schaudinn¹ asserted that after tertian malarial infections had persisted for some time, the microgametocytes gradually disappeared from the peripheral blood, until finally only macrogametes were found. In estivo-autumnal infections I have observed just the opposite, the microgametocytes being the last to disappear from the peripheral blood. It is questionable, however, as to how much weight should be placed on the

Fig. 1.—A young macrogamete of *Plasmodium vivax* (the tertian plasmodium). Note the darker color and the larger amount of pigment than is present in the microgametocytes shown in Figure 2; the larger size of the organism; and the more distinct outline, showing more intense staining. $\times 1200$.

relative proportion of the gametes as observed in the peripheral blood, for it is probable that it is not a true index of the exact proportion present in the body.

THE MORPHOLOGY OF THE SEXUAL FORMS OCCURRING IN MAN

In considering the morphology of the sexual forms it is necessary to describe the gametes of each species, as observed in both fresh and stained specimens of blood. The description given of the morphology of the various forms will include only the salient diagnostic points, and will be found true of the vast majority of the plasmodia examined, although frequent deviations will be observed, due to pressure in preparing the specimen or artefacts produced during the staining process. The stain which I used was Wright's modification of the Romanowsky method.

THE SEXUAL FORMS OF *PLASMODIUM VIVAX* (THE TERTIAN PLASMODIUM)

Fresh Preparations.—In fresh preparations it is practically impossible to distinguish the gametes of *Plasmodium vivax* from the schizonts (forms of the human cycle) until they are fully developed. While individual gametes may contain a larger amount of pigment, which is coarser in structure, than do the schizonts, this distinction cannot always be made; while the hyaline stage cannot be distinguished by the loss of the "ring-form," as this form is frequently absent in tertian infections in which gametes have not developed. When the gametes are fully developed, however, they may be easily distinguished from the fully developed schizont, and the male and female forms may be readily differentiated.

Fig. 2.—Two young microgametocytes of *Plasmodium vivax*. Note the lighter color and smaller amount of the pigment, and the less distinct outline, showing the poor staining quality of this form of the plasmodium. $\times 1200$.

The Living Macrogamete.—The tertian macrogamete, when fully developed, measures from 9 to 11 microns in diameter, and is perfectly circular in shape; the protoplasm appears more granular than that of the mature schizont; while the pigment, instead of being distributed throughout the protoplasm, is arranged in the form of very large grains, clumps or rods, about the periphery of the organism, or in a wreath-like manner at some distance from the periphery. The pigment is not motile and the organism appears more refractile than the schizont. Very rarely a flagellum or microgamete may be observed attached to this form of the parasite, but the character of the movements of the flagellum and the peculiar arrangement of the pigment within the macrogamete serve to distinguish it from a flagellating microgametocyte.

The Living Microgametocyte.—The microgametocyte of *Plasmodium vivax* is slightly smaller than the macrogamete, measuring, when fully

developed, from 8 to 10 microns in diameter; it is spherical in shape, the protoplasm is less granular, and the pigment is generally distributed throughout the protoplasm. The pigment is larger in amount than in the macrogamete and is sluggishly motile when the organism has just been liberated from the red corpuscle in which it has developed. In the fully developed form the pigment may be immotile, and in such instances, instead of being collected in clumps about the periphery of the red cell, or in a wreath-like manner near the center, as in the macrogamete, it is scattered in minute clumps throughout the protoplasm.

The phenomenon which distinguishes the living microgametocyte from all other forms of malaria plasmodia is the process known as flagel-

Fig. 3.—A fully developed macrogamete of *Plasmodium vivax*. Note dark, rod-like pigment granules, the chromatin collected in a dense mass at one side of the organism, and the distinct appearance of the organism due to the deep staining of the protoplasm. $\times 1500$.

lation, during which the microgametes, which have developed within the microgametocyte, are finally extruded and liberated from the parent body. The microgametocytes which are about to undergo this change are easily recognized because of the violent activity of the pigment within them, and the undulatory movements of the border of the parasite. This violent activity of the pigment is due to the flagella or microgametes moving about within the parent body, and if such an organism be carefully watched it will be observed that eventually a number of delicate filaments suddenly make their appearance at the periphery of the organism and lash about in the blood plasma. These are the microgametes and vary in number from two to four. They are perfectly hyaline in appearance, although not infrequently a few granules of pigment may be observed within them, derived from the microgametocyte. Before the occurrence

of flagellation the pigment of the microgametocyte tends to collect toward the center of the organism and is arranged in a more or less compact mass.

After lashing about for a variable time, one, or perhaps all, of the microgametes succeed in freeing themselves from the parent body, and disappear among the red blood corpuscles. In rare instances one of these free microgametes may be observed attached to a macrogamete, but this is altogether exceptional, and such bodies should be distinguished from a flagellating microgametocyte.

The Living Microgamete.—In fresh blood preparations the microgamete appears as a thread-like, hyaline body, which possesses a serpentine motility, enabling it to progress among the red blood corpuscles which are moved about during its passage. It is often discovered in fresh prep-

Fig. 4.—A fully developed microgametocyte of *Plasmodium vivax*. Note the fine pigment granules, the larger amount of chromatin, arranged in a loose network, instead of in a solid mass, as in the macrogamete, and the indistinct appearance of the organism due to its poor staining quality. $\times 1500$.

arations by the very apparent movement of the erythrocytes in its vicinity. As a rule, the microgametes are very slender bodies, but sometimes short stout forms are observed, the significance of which has not been determined. Very frequently one extremity of the microgamete is clubbed, while the opposite extremity is sharply pointed, but usually after liberation both extremities are sharply pointed. The length of the microgamete varies, the usual measurement being from 16 microns to 20 microns, but forms have been observed measuring as much as 40 to 50 microns, or even more. A few pigment granules may sometimes be observed within the microgametes and this pigment is apparently rapidly extruded, for no pigment is ever observed in microgametes which have

become attached to macrogametes. The variation in the length of the microgametes is more marked in the tertian species than in the quartan or estivo-autumnal species, and short, rather thick forms are frequently observed along with long slender forms, in infections with this parasite.

Stained Preparations.—Unlike fresh preparations, in stained preparations the young intracorpuseular stages of the tertian gametes may be recognized. This is true of all but the very earliest intracorpuseular stage, in which the gamete is with great difficulty differentiated from the schizont. While this is true, it is possible, even before the development of pigment, to differentiate the gametes by their staining reactions. When stained by the Wright method all forms of the malarial plasmodia present the same fundamental staining reactions, but differences are observed in the intensity of the reaction in the various stages of growth, and in the

Fig. 5.—A macrogamete of *Plasmodium falciparum* (the tertian estivo-autumnal plasmodium). Note the slender form of the crescent, which is characteristic of the macrogametes. $\times 1500$.

forms concerned in sporogony and schizogony. The protoplasm stains blue; the chromatin of the nucleus a bright red or violet; while the achromatic portion of the nucleus remains unstained. The only variation which occurs in the staining reaction of the gametes consists in the degree of color imparted by the stain to the various forms. The tertian gametes when stained by this method consist of a mass of blue protoplasm, enclosing the red-stained chromatin; the achromatic portion of the nucleus is not always distinct, and the young gamete is considerably larger than the young schizont. The pigmented gamete contains more pigment than do the developing schizonts, while the chromatin shows no evidence of division and distribution throughout the protoplasm, being arranged in small irregular masses confined to one portion of the organism, or broken up

into fine fibrils, surrounded by an unstained area, which is again surrounded by the blue-stained protoplasm. While intracorpuseular, the gametes are always spherical in shape, but are sometimes distorted during the preparation of the specimen. The following data are of service in differentiating tertian gametes from tertian schizonts:

1. The young, intracorpuseular gamete is never "ring"-like in shape, as the achromatic zone is imperfectly defined.
2. The gamete is larger than the corresponding stage in growth of the schizont.
3. The pigment in the gamete is larger in amount and earlier developed.
4. The chromatin is arranged in a loose skein or in minute masses, and is not distributed throughout the protoplasm at any stage in the development of the gamete.

Fig. 6.—Two microgametocytes of *Plasmodium falciparum*. Note the plump kidney shape which is characteristic of the microgametocytes of the estivo-autumnal plasmodia. $\times 1500$.

5. When sporulating parasites are present the gametes are easily recognizable, for, though larger than the sporulating bodies, they present no evidence of segmentation of the chromatin.

The Stained Macrogamete.—In stained specimens the tertian macrogamete stains a very intense blue, and the intensity of the staining serves to distinguish this form of the plasmodium from the schizont or the microgametocyte. The chromatin, in the earliest stage of development, consists of a small dot situated near the center of the organism, while in later stages of development several dots or rods of this substance are present, situated near the periphery of the organism, but it is not distributed throughout the protoplasm. The chromatin is comparatively small in

amount and stains a brilliant crimson. The pigment is almost black in color in stained preparations and arranged in irregular masses near the periphery or in a wreath-like manner about the center of the parasite. The pigment is usually in the form of rods, measuring from 1 to 3 microns in length.

The Stained Microgametocyte.—In stained specimens the microgametocytes of *Plasmodium vivax* vary in appearance during the various stages of development. The young microgametocytes stain a very pale blue and contain a comparatively large dot of intensely stained chromatin. The fully developed microgametocytes present a very poorly stained protoplasm, the blue tinge being so faint in many instances as to be distinguished with difficulty, the protoplasm appearing hyaline. The chromatin is always large in amount in all stages of development and stains an intense red. In microgametes which are about to liberate flagella, the chromatin is observed to be divided into from four to eight masses, which are arranged about the periphery of the organism, and these forms are sometimes mistaken for sporulating quartan plasmodia. The pigment is larger in amount than in the schizonts or the macrogametes, and stains a greenish blue color. It is in the form of fine grains or short very slender rods. If microgametocytes which have liberated the microgametes or flagella be studied in stained specimens before the microgametes have separated from the parent body, it will be observed that the chromatin of the parent body may be traced into the flagella. In some instances it will be seen that all of the chromatin has become collected in the microgametes, the microgametocyte being free from this substance, although in most instances a few irregular clumps, minute in size, may still be observed within it. It is evident from this observation that most of the chromatin of the microgametocyte is expended in the formation of the microgametes.

The chromatin in the microgametocytes is always larger in amount than in the macrogametes, and is collected in the form of rather thick febrils arranged in irregular masses in the protoplasm, the whole being surrounded by an achromatic zone. It is never distributed in regular masses throughout the protoplasm as it is in the schizonts prior to or during sporulation. In stained specimens the shape of the microgametocyte is oval or circular, but it may be distorted during the preparation of the specimen.

Differentiation of the Tertian Microgametocyte from the Macrogamete.—The microgametocyte of *Plasmodium vivax* may be readily differentiated from the macrogamete by attention to the following points:

1. The microgametocyte is smaller than the macrogamete.
2. In fresh specimens the microgametocyte has a greater amount of pigment than the macrogamete and the peculiar "swarming motion" of the pigment serves to differentiate this form of the plasmodium from the full-grown schizont, in which the pigment is motionless; and from the macrogamete, in which the pigment is arranged in a wreath-like form and is either motionless or very sluggishly motile. The pigment in the microgametocyte is in the form of fine granules or very short slender rods, while in the macrogamete the pigment is in the form of large thick rods.
3. The protoplasm in living specimens of the microgametocyte is less granular in structure.
4. The presence of the microgametes or flagella in the microgametocyte and the process of flagellation. While flagella may very rarely be seen attached to macrogametes, it is generally easy to distinguish this form because of the peculiar character of the motility of the microgamete in its endeavor to penetrate the macrogamete.
5. In stained specimens the greenish-blue stain of the protoplasm in the microgametocyte serves to distinguish it from the macrogamete, in which the protoplasm is stained a deep blue.
6. The microgametocyte has a larger amount of chromatin, collected in masses, the whole surrounded by an achromatic zone, while in the macrogamete the chromatin is small in amount and is in the form of minute dots or very delicate fibrils, collected near the periphery of the parasite, surrounded by an achromatic zone.

The Stained Microgametes.—In stained preparations the microgametes or flagella of *Plasmodium vivax* are observed to be composed of a slender lengthened mass of protoplasm which stains a pale blue and which contains a certain amount of chromatin, stained a brilliant crimson. The chromatin may be situated near the center of the organism in the form of a more or less regular mass, or it may be distributed throughout the protoplasm in the form of minute granules, or extend in the form of a broken thread along the greater portion of the length of the microgamete. Before liberation from the microgametocyte the microgametes often present a free clubbed extremity, but after they have become detached from the parent organism the extremities are always pointed. In general appearance the microgametes resemble the spirochetes, but they are not regularly curved and, of course, the staining reactions, and the more delicate appearance of the microgametes serves to distinguish them from the latter. The chromatin is very large in amount in the microgamete and not infrequently organisms are observed in which it is very difficult to distinguish any proto-

plasm, the entire organism appearing to be composed of chromatin. For this reason the microgametes are often overlooked in the examination of malarial blood, but if attention is paid to this point it will be found that not a few stained preparations contain this form of the organism.

THE SEXUAL FORMS OF *PLASMODIUM MALARIAE* (THE QUARTAN
PLASMODIUM)

Fresh Preparations.—The gametes of *Plasmodium malariae* are very similar to those of *Plasmodium vivax*, and for this reason will be described but briefly. In their early stages of development they cannot be distinguished from the schizonts, in fresh blood, but when they are fully developed they may be distinguished by their larger size, the absence of sporulation, and the greater amount of pigment present.

The Living Macrogamete.—The quartan macrogamete is larger than the schizont when fully developed and differs from the latter in possessing a more granular protoplasm and a greater amount of very coarse pigment. We cannot depend on the motility of the pigment and its arrangement in differentiating this form of the quartan plasmodium, for in the quartan schizont the pigment is often observed to be arranged in large clumps about the periphery; but a well-marked wreath-like arrangement of the pigment about midway between the center of the organism and the periphery is very characteristic of the macrogamete. The process of flagellation is never observed in this form of the organism.

The Living Microgametocyte.—The description of the microgametocyte of *Plasmodium vivax*, already given, applies equally well to the microgametocytes of *Plasmodium malariae*. We observe the same development of microgametes or flagella; the same relative proportion of pigment; and the same peculiar "swarming" motility in this pigment. In size the quartan microgametocyte is slightly larger than the schizont. The pigment is coarser in character than in the tertian microgametocyte.

The Living Microgamete.—The microgamete of the quartan plasmodium is smaller than that of the tertian, but is otherwise similar. I believe that if it were possible to compare the two forms in fresh specimens, the quartan form would be found to be considerably thicker than the tertian, and less actively motile. Aside from the difference in length, which may amount to several microns, it is impossible to distinguish the tertian from the quartan microgamete.

Stained Preparations.—In stained preparations the gametes of *Plasmodium malariae* resemble very closely those of the tertian plasmodium, though they are smaller and stain more intensely. In their earliest intra-

corpuscular stages of development they may be distinguished from the schizont by the situation of the chromatin dot within the ring of protoplasm, and the absence of a distinct achromatic zone. When fully developed they may be distinguished by their larger size; the difference in their staining reactions; the absence of indications of sporulation; and the arrangement and amount of the chromatin.

The Stained Macrogamete.—The protoplasm of the macrogamete stains a deep blue and the chromatin a dark crimson. When fully developed the macrogamete measures from 10 to 12 microns in diameter, so that it is considerably larger than the fully developed schizont; the chromatin is collected at some portion of the periphery in the form of fine fibrils or small masses, surrounded by an unstained space; the pigment is coarse and is distributed throughout the protoplasm, and there is never any indication of the division of the chromatin, so characteristic of the schizont when it has reached the same size. The shape of the organism is always oval or circular when the smears have been carefully prepared.

The Stained Microgametocyte.—The protoplasm of the quartan microgametocyte stains a pale blue which serves to distinguish it very readily from the deep blue macrogamete. The chromatin is large in amount, stains a deep red or violet, and is arranged in a loose skein, composed of definite fibrils or granules, situated to one side of the center of the organism. It never shows the evidences of division characteristic of the chromatin in sporulating parasites. The quartan microgametocyte is much smaller than is the tertian and the staining reactions are more pronounced.

The Stained Microgamete.—The microgametes of *Plasmodium malariae* are indistinguishable in stained preparations from those of *Plasmodium vivax*, and the description already given of the latter answers equally well for the microgametes of this species.

THE SEXUAL FORMS OF THE ESTIVO-AUTUMNAL PLASMODIA

The sexual forms of *Plasmodium falciparum* (the tertian estivo-autumnal plasmodium) and of *Plasmodium falciparum quotidianum* (the quotidian estivo-autumnal plasmodium) are commonly known as "crescents," and these are the only malarial gametes having a crescentic shape. For this reason, when fully developed they are very easily differentiated from the estivo-autumnal schizonts, and from the tertian and quartan gametes. In the young intracorporeal stage of development the crescentic shape is not present, and at this time the estivo-autumnal gametes are indistinguishable from those of the quartan species. When fully developed the male and female gametes are very easily distinguished

from one another. It should be remembered that the presence of crescentic gametes invariably indicates an estivo-autumnal infection, and further, that such infection is transmissible to the mosquito.

The differences between the gametes of the tertian and quotidian estivo-autumnal plasmodia consist chiefly in variation in size and shape, the general morphology of both species being identical. For this reason they will be considered together, the salient points of difference being spoken of in the general description.

The estivo-autumnal gametes, like those of *Plasmodium vivax* and *Plasmodium malariae*, develop within the red corpuscles, from which they are finally liberated by the degeneration of the latter. The microgametocytes then undergo flagellation and produce the microgametes, the crescentic microgametocyte having previously become oval and then spherical in shape. The macrogametes also become oval and spherical in shape before fertilization by the microgamete, and it is probable that important maturation changes occur in this form during this period of development.

Fresh Preparations.—The earliest stage in the development of the estivo-autumnal gamete cannot be differentiated from the young schizont, but after the formation of pigment the gametes are distinguished by a larger amount of the latter; their ovoid or crescentic shape, even in early stages of development within the red corpuscle; and their slighter degree of ameboid activity. The protoplasm of the gametes, especially of the macrogametes, is more granular in structure; the pigment is darker in color, and the organism is more refractive and sharply outlined. As development proceeds the infected red cell shrinks about the gamete, forming an envelope, which is very noticeable between the poles of the crescent, where it projects and forms what is known as the “bib” of the crescent.

The Living Macrogamete.—The macrogamete of *Plasmodium falciparum*, when fully developed, measures from 11 to 15 microns in length, and from 3 to 5 microns in breadth, while that of *Plasmodium falciparum quotidianum* measures from 8 to 9 microns in length. In both species the macrogametes are distinguished from the microgametocytes by their more slender form and the arrangement of the pigment. The protoplasm of the estivo-autumnal macrogametes is very opaque and granular in appearance, even in early stages of the development of the organism within the red corpuscle. The pigment is dark brown in character, and after the destruction of the infected red cell is arranged in a dense mass or in a wreath-like manner, at or near the center of the organism. The fully developed macrogamete possesses a clearly cut, single outline; a very granular, opaque protoplasm, and a considerable amount of rod-like pig-

ment, situated near the center of the crescent. Preparatory to fertilization the crescentic form is lost, the macrogametes first becoming oval in outline, and then circular, while the pigment becomes divided into small clumps arranged in a perfect circle surrounding the center of the parasite. Reasoning from analogy, it is probable that during this change in shape important changes are occurring in the nuclear chromatin, in the nature of reduction phenomena. Such changes have not as yet, however, been demonstrated. While the production of the ovoid and round forms from the macrogamete occurs normally within the middle intestine of the mosquito, the process is very frequently observed in blood which has been removed for some time from the body. The quotidian estivo-autumnal macrogamete is smaller than the tertian form; has a smaller amount of pigment, which is granular rather than rod-like in structure; a less granular protoplasm; while the extremities of the crescent are pointed instead of rounded, as they are in the tertian estivo-autumnal macrogamete.

The Living Microgametocyte.—The microgametocyte of *Plasmodium falciparum*, when fully developed, measures from 7 to 10 microns in length, and from 4 to 6 microns in breadth, while that of *Plasmodium falciparum quotidianum* measures from 6 to 7 microns in length and is slightly broader than the tertian microgametocyte. Both are kidney-shaped rather than crescentic, a fact which renders their differentiation from the estivo-autumnal macrogametes a matter of little difficulty. The protoplasm is less opaque and granular than that of the macrogametes, while the pigment is in finer particles and may be distributed throughout the protoplasm of the crescent or even collected in clumps at one pole of the crescent. The pigment is generally sluggishly motile, while the parasites are still intracellular and become very actively so just prior to flagellation.

After the total destruction of the red corpuscle in which they have developed, the estivo-autumnal microgametocytes flagellate in the same manner as do the microgametocytes of the tertian and quartan plasmodia. The kidney shape is lost, the organism becoming ovoid and round; the pigment becomes very motile; the protoplasm appears violently agitated; and suddenly the microgametes are extruded as very delicate, motile filaments, numbering from two to four, and, rarely, even more. After lashing about for a while the microgametes become detached from the parent body and disappear among the red corpuscles. This process occurs naturally in the middle intestine of the mosquito, but may frequently be witnessed in blood containing microgametocytes, after it has been removed for some time.

The quotidian estivo-autumnal microgametocyte differs from that of the tertian estivo-autumnal species in the following particulars: smaller size, seldom measuring over 7 microns in length; the smaller amount of pigment; and the very plump shape of the organism, closely resembling that of a lima-bean.

The Living Microgametes.—The microgametes of the estivo-autumnal plasmodia are indistinguishable from those of the tertian and quartan plasmodia, consisting of delicate, motile filaments, resembling spirochetes in fresh blood preparations. We possess no data which enable us to differentiate the microgametes of the various species of the malarial plasmodia from one another in either fresh or stained material.

Stained Preparations.—In stained preparations the estivo-autumnal gametes are easily differentiated from the schizonts, even in an early stage of development. Before the development of pigment the gametes may be distinguished from the schizonts by their spherical shape and the lack of the “ring” form so characteristic of the estivo-autumnal schizont. At this stage of development the gamete consists of a circular portion of protoplasm, which stains blue, enclosing a dot or minute clump of chromatin, stained a deep red or violet. After the development of pigment the gametes are distinguished by their ovoid or crescentic shape, even while contained in the red corpuscle; the presence of a larger amount of chromatin; and the presence, in properly stained specimens, of a deep red band encircling the parasite. When fully developed the gametes are most easily distinguished by their crescentic shape.

The Stained Macrogamete.—The staining reactions of the estivo-autumnal macrogametes are the same as those of the tertian and quartan forms, i. e., the protoplasm stains a deep blue and the chromatin a brilliant red or violet. The protoplasm stains most intensely in the young intracellular macrogametes, and this is also true of the chromatin. When fully developed the protoplasm of the crescentic macrogamete stains deeply blue, the color being most intense at the poles of the crescent; not infrequently deeply stained areas are observed, especially numerous near the poles of the crescent. The chromatin is situated at or near the center of the macrogamete, stains a light red or pink, and is not infrequently obscured by the pigment, which being collected at the center of the organism appears to be mixed with the chromatin. In many instances the chromatin is observed to lie at the center of the crescent, surrounded by a perfect ring of pigment granules. Many interesting variations are noted in the staining reactions of the macrogametes, most of them due to pressure during the preparation of the specimen, or to artefacts produced during the staining process.

The remains of the red blood corpuscle surrounding many of the macrogametes stains a salmon or pink color, thus forming a smooth pink border to the crescent, or this border may be broken and jagged in appearance, or missing at some portion of the periphery of the crescent. The so-called "bib" of the crescent is often beautifully shown in stained specimens, consisting of a faintly stained pink mass lying within the concavity of the crescent, the projecting border staining a deeper pink or red. Such gametes are still intracorpuseular, although the crescentic shape is fully developed.

Some of the macrogametes, which are apparently extracorpuseular, present a very deeply stained red border, the nature of which is still uncertain. A similar red border is not infrequently observed surrounding the young intracorpuseular gametes, so that it is not probable that it is due to the substance of the infected red corpuscle. In addition, the staining reaction is different, the protoplasm of the red corpuscle staining a light red or pink, while the edge of the gamete stains a deep red, very similar to the staining reaction observed in the chromatin.

The following features serve to distinguish the estivo-autumnal macrogametes from the microgametocytes in stained preparations:

1. The long slender shape of the crescents.
2. The character of the chromatin which is situated at or near the center of the crescent in a dense mass or irregular clump.
3. The deep blue staining of the protoplasm.
4. The concentration of the pigment at or near the center of the organism, in the form of masses, or in wreath-like manner, surrounding the chromatin.

The Stained Microgametocyte.—The protoplasm of the microgametocyte stains a delicate robin's-egg blue, very different from the deep blue or purple of the macrogamete. In some instances the protoplasm refuses to stain. The chromatin is arranged in the form of a loose network composed of delicate fibrils and granules which stain a deep pink or light red. Sometimes the chromatin appears to be divided into several clumps distributed throughout the protoplasm. The pigment is distributed throughout the protoplasm, stains a greenish brown, and is in the form of fine granules or very short rods. Some portions of the microgametocytes stain more intensely than others, giving the crescent a peculiar blotchy appearance, and sometimes only the border of the crescent takes the stain. The chromatin not infrequently stains so poorly as to be barely distinguishable. The remains of the red blood corpuscle, in which the microgametocyte has developed, stains in the same manner as described for the macrogamete. The chromatin of the ovoid and round forms,

which develop from the microgametocyte, is arranged in small clumps about the periphery and stains a deeper red than while the organisms are crescentic in shape.

The points already mentioned in distinguishing the tertian estivo-autumnal microgametocyte from the quotidian are observed in stained specimens of blood, and, in addition, it is found that the quotidian microgametocyte contains a larger amount of more intensely stained chromatin, while the protoplasm also stains a deeper blue than does that of the tertian estivo-autumnal microgametocyte.

The estivo-autumnal microgametocyte may be readily differentiated from the macrogamete by attention to the following points:

1. Shape: The plump kidney shape.
2. Staining reaction: The pale blue staining of the protoplasm and the less intense staining of the chromatin.
3. Chromatin: The arrangement of the chromatin in the form of a loose network.
4. Pigment: The distribution of the pigment throughout the protoplasm.
5. Size: The microgametocytes are shorter and broader than are the macrogametes.

The above description of the morphology of the sexual forms of the malarial plasmodia observed in fresh and stained specimens of human blood includes only those points which are of importance in diagnosis; many minute details of the structure of these interesting bodies have not been touched on for the reason that they can be studied only where a large amount of material is available, are not of importance from a diagnostic standpoint, and are not of constant occurrence. It is believed that attention to the differential details described in this paper should enable any one, with a little practice, to be able to diagnose these forms.

PERCENTAGE OF PATIENTS IN WHOM GAMETES OCCUR

As only those individuals in whom the sexual forms, or gametes, occur are capable of transmitting malaria to the mosquito, and thus, indirectly, to man, it is of interest to know how large a percentage of our malarial patients become "carriers." The number will vary, of course, in different localities, owing to conditions favoring the persistence of the infection; the type of infection present; the thoroughness with which treatment is carried out; and perhaps other conditions with which we are as yet unfamiliar. This subject has been most thoroughly studied in estivo-autumnal infections because of the ease with which the crescentic gametes are recognized. The data we possess show beyond question that the percentage of

patients having gametes in their peripheral blood varies considerably in different localities and that the incidence of gametes in the peripheral blood cannot be taken as a true index of their actual occurrence in the body, for in many instances it has been demonstrated that blood obtained by splenic puncture will be found full of gametes when none can be found in the peripheral blood. Rogers⁴ observed crescents in only 10 per cent. of the cases he studied in Europe, while Deaderick⁵ states that in his experience they are very rarely observed in the peripheral blood. In my experience gametes have been observed in a little over 33 per cent. of my cases of estivo-autumnal infections. A longer search might have considerably increased this percentage, for it often requires the patient examination of several specimens of blood to demonstrate the presence of crescents in estivo-autumnal infections. In the instances of recurrent infection in Filipinos whom I studied, to whom no quinin had been administered for weeks or months, crescents occurred in fully 80 per cent. of the infected individuals, and were as numerous in the adult Filipinos as in the children. Manson⁶ believes that the estivo-autumnal gametes are more frequently observed in patients who have contracted their malaria in the tropics and have returned to temperate regions, and that gametes are more rarely observed in the tropics. I cannot confirm this opinion, as in my experience as large a proportion of patients in the Philippines, if untreated, show gametes as in the United States. Soldiers returning from Cuba and the Philippines, and suffering from recurrent malaria after their return, did not show a larger percentage of infections with crescents than soldiers serving in those countries and suffering from malaria while there, and it is not at all uncommon to observe crescents in the peripheral blood of the Filipinos.

From what has been said it is evident that the percentage of cases showing estivo-autumnal gametes varies considerably, but it may be said, without danger of contradiction, that a large majority of our patients will develop these bodies unless they are properly treated with quinin. I believe, from my observations on a native race, the Filipinos, that at least 80 per cent. of our estivo-autumnal patients will become carriers of this type of infection unless thoroughly treated.

We possess but very little data concerning the percentage of patients infected with the tertian and quartan plasmodia in whom gametes develop.

---

4. Rogers, L.: *Malarial Remittent Fevers*, Jour. Trop. Med. and Hyg., London, 1903, Sept. 1, p. 272.

5. Deaderick, W. H.: *A Practical Study of Malaria*, Ed. 1, Philadelphia, 1909, W. B. Saunders & Co.

6. Manson, Sir P.: *Tropical Diseases*, Ed. 4, New York, 1907, William Wood & Co.

I have found that about 50 per cent. of tertian and quartan infections which I observed showed a few gametes in the peripheral blood, provided the infections had persisted for some time and quinin had not been administered during the first week or two of the infection. Here, again, it is not safe to infer that no gametes are developed because they may not be demonstrable in the peripheral blood, as it is probable that in these infections, as in the estivo-autumnal, gametes would be often found in the spleen and bone-marrow.

While the absence of gametes from the peripheral blood would appear to demonstrate that the individual is not a carrier of malarial infection, and while, for practical purposes, such individuals have to be considered as safe in this respect, it does not follow that such is really the case, for the gametes may be present in the internal organs and find their way into the peripheral circulation at intervals in sufficient numbers to infect mosquitoes, or they may be present in such small numbers in the peripheral blood as to render their demonstration impossible, and yet the blood may be infective. As regards the latter statement it should not be forgotten that other blood protozoa, such as the trypanosomes and spirochetes, often occur in numbers so small in the peripheral blood as to render their demonstration impossible, although the blood is infective to animals. A great deal of work needs to be done on this subject before we can be sure of our premises, and the recent experiments of Darling⁷ are a step in the right direction. His observation that the peripheral blood must contain a certain number of gametes in order to be capable of infecting the mosquito is very suggestive, but needs confirmation by a large number of experiments.

THE TIME OF OCCURRENCE OF THE SEXUAL FORMS

It is universally admitted that gametes do not develop until the malarial infection has persisted for several days, uninfluenced by quinin. The length of time required for the development of the gametes of the estivo-autumnal plasmodia varies from eight to fifteen days, the usual period being about twelve days. In other words, an estivo-autumnal infection must have persisted for nearly two weeks before we can expect to find crescents in the peripheral blood. The gametes of *Plasmodium vivax* and *Plasmodium malariae* appear in the peripheral blood in from seven to ten days after the onset of definite symptoms of infection.

It should be remembered that the malarial infection may have been present for a considerable time before the occurrence of definite symptoms,

---

7. Darling, S. T.: Transmission of Malarial Fever in the Canal Zone by Anopheles Mosquitoes, Jour. Am. Med. Assn., 1909, liii, 2051.

so that the actual time required for the development of gametes may be much longer than is generally supposed from our examinations of the peripheral blood after the occurrence of symptoms. Again, the blood may contain gametes before any symptoms of malarial infection have been noted. I have observed gametes in cases of latent malaria in which no malarial symptoms had ever occurred, so that we may find these sexual forms present on the very first day of an obvious infection.

The fact, however, that several days elapse after the occurrence of symptoms before gametes appear in the peripheral blood, in the vast majority of patients, is of the greatest importance from a prophylactic standpoint, as will be mentioned later. It is also very significant as regards the origin of these bodies, and appears to indicate that they are not introduced into man by the mosquito, but that, as Schaudinn¹ believed, they are differentiated during the human life cycle of the plasmodia as the result of the reaction of the human system to the plasmodia. Certainly all of the evidence we possess points to this conclusion.

THE EFFECT OF QUININ ON THE SEXUAL FORM

The consensus of opinion is that after the gametes of the various species of plasmodia are fully developed quinin has no action on them, so far as their development within the mosquito is concerned. In the early intra-corporal stages I have observed marked changes in their staining reactions which lead me to believe that during this period in their development quinin is capable of injuring them and preventing their full development, for the protoplasm and chromatin stains atypically and the latter is reduced in amount. If quinin be given promptly during the initial attack of fever it will effectually prevent the development of gametes in the vast majority of infections; in those cases in which it does not do so it is probable that the infection has been present for some time in a latent state and that gametes have developed before the appearance of symptoms.

The experiments of Schoo,⁸ while not confirmed, appear to indicate that the tertian gametes may sometimes be prevented from development in the mosquito by the administration of quinin. He found that in two tertian cases, the patients being bitten by *Anopheles* before the administration of quinin, nearly all of the insects became infected, while the same individuals, bitten after the administration of this drug, were unable to infect any of the insects. These observations are at variance with those

---

8. Schoo, F.: Wat kan er aan Prophylaxis der Malaria in Nederland gedaan worden? Nederl. Tijdschr. Geneesk., 1902, xvii, 974.

of many investigators and should be carefully repeated. The fact that no morphologic changes occur in the fully developed tertian gametes after the administration of quinin is pretty good evidence that the drug cannot affect them so seriously as to prevent their further development in the mosquito.

No morphological changes occur in the fully developed quartan or estivo-autumnal gametes after the administration of quinin to patients in whom these forms are present. The estivo-autumnal crescents are capable of development within the mosquito even though the drug has been administered for weeks, as has been proven by Marchiafava,⁹ Gualdi,¹⁰ Martirano,¹¹ Darling⁷ and others. On the Isthmus of Panama Darling⁷ found that the development of malarial gametes in the middle intestine of *A. albimanus* was not affected by quinin "when such an infected mosquito feeds daily or on alternate days for fifteen days on patients who are receiving 30 grains of quinin in solution daily. In these instances the zygotes mature and sporozoites reach the salivary glands in the usual period."

The observations of Darling prove that the administration of quinin to patients whose blood contains tertian and estivo-autumnal gametes results in the gradual disappearance of these forms from the peripheral blood. He found that the administration of 30 grains of the drug in solution per day was capable of reducing the number of crescents in the peripheral blood from 67 per 100 leucocytes to 1 per 200 leucocytes in twenty-five days, while in another case the crescents were reduced from 92 per 100 leucocytes to 1 per 100 leucocytes in fifteen days. On the other hand, in cases in which quinin was not administered the crescents appeared to increase rather than diminish in the peripheral blood. As the result of these experiments Darling concludes that the administration of quinin in gamete-carriers is of the greatest importance in the prophylaxis of malarial disease.

NUMBER OF GAMETES NECESSARY FOR INFECTION OF THE MOSQUITO

It is but reasonable to believe that the peripheral blood must contain a certain number of gametes in order to render it infective to the mosquito, but I know of only one investigator who has undertaken to prove

9. Marchiafava, E.: *Malaria*, Twentieth Century Practice, xix, Ed. 1, New York, 1900, William Wood & Co.

10. Gualdi, T., and Martirano, F.: *L'azione della chinina sulle semilune*, Ann. d. ig. sper., 1901, xi, 6.

11. Martirano, F.: *Ann. d. ig. sper.*, 1901, xi, 6.

the number necessary for such infection. In a very ingenious series of experiments Darling⁷ has arrived at the conclusion that the peripheral blood must contain at least 12 crescents per cubic centimeter or more than one per 500 leucocytes in order to be infective to the mosquito, and that patients whose blood contains this number should be regarded as gamete-carriers and kept in hospital until, by the administration of quinin, the number is reduced below this minimum. Darling arrived at his results by comparing the number of crescents present with the number of leucocytes, estimating the amount of blood ingested by the mosquito in biting by weighing the insect both before and after eating, and then calculating the number of crescents contained in the amount of blood ingested. While the results of these experiments are very suggestive, it should be remembered that many factors have to be considered in making such comparisons. The presence of a slight or marked leucocytosis; local congestions; factors determining the number of gametes present in the peripheral blood and in the internal organs; all have to be carefully weighed in reaching a true understanding of the significance of such investigations.

As regards the reduction in the number of gametes in the peripheral blood after the administration of quinin, we should not be too hasty in concluding that such reduction means the destruction of these bodies, for it is much more probable that they are simply driven from the peripheral circulation to the spleen and bone-marrow, for we possess no data showing that quinin produces any changes whatever in the morphology of the fully developed gametes. The disappearance of the gametes from the peripheral blood may, of course, render the blood non-infective for the time being, but we do not know how long a period elapses before the gametes may reappear in the peripheral circulation and the blood become infective. It appears to me that, while it may be good practice to keep our malarial patients in the hospital until the gametes are reduced to the number mentioned by Darling, it would be better to keep them until the gametes entirely disappear from the peripheral blood. Even then we should not be too sanguine as regards the future non-infectivity of such patients for many malarial patients relapse in whom no gametes can be demonstrated in the peripheral blood.

CONCLUSION

From what has been said regarding the action of quinin on the malarial gametes it is evident that if we thoroughly treat our malarial patients during the initial attack of the disease we shall prevent the formation of

these bodies and, therefore, the transmission of the infection to the mosquito. A great deal of the malaria prevalent in every malarial locality is directly traceable to improperly treated patients, and the prophylaxis of the disease will be immensely helped by the thorough treatment of every infection. The practice of regarding such infections as cured because the active symptoms have disappeared is a most common and a most pernicious one, and one that is responsible for the transmission of a very large proportion of malarial disease. Every malarial patient should be kept on quinin for at least a week or two after the disappearance of symptoms, and should take the drug, in 10-grain doses, once a week for two months after the acute attack.